4-Methoxyphenyl 4-(3,4,5-Trimethoxybenzyl)-1-Piperazineacetate Monofumarate Monohydrate (KB-5492), a New Anti-Ulcer Agent with a Selective Affinity for the Sigma Receptor, Prevents Cysteamine-Induced Duodenal Ulcers in Rats by a Mechanism Different from That of Cimetidine

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ABSTRACT—Both KB-5492, a new anti-ulcer agent, and cimetidine, administered orally at 25–200 mg/kg, dose-dependently prevented cysteamine (400 mg/kg, s.c.)-induced duodenal ulcers in rats with ED₅₀ values of 63 and 40 mg/kg, respectively. Anti-ulcer doses of cimetidine, but not KB-5492, inhibited gastric acid hypersecretion induced by cysteamine (400 mg/kg, s.c.). In contrast, anti-ulcer doses of KB-5492, but not cimetidine, increased duodenal HCO₃⁻ secretion in normal anesthetized rats. These findings suggest that KB-5492 prevents cysteamine-induced duodenal ulcers by stimulating duodenal HCO₃⁻ secretion, whereas cimetidine does so by inhibiting cysteamine-induced gastric acid hypersecretion.

Keywords: KB-5492, Ulcer (cysteamine-induced), Bicarbonate secretion

KB-5492, 4-methoxyphenyl 4-(3,4,5-trimethoxybenzyl)-1-piperazineacetate monofumarate monohydrate, is a new anti-ulcer agent previously shown to prevent various experimental gastric mucosal lesions in rats (1, 2). Although KB-5492, at anti-ulcer doses, did not affect either basal or histamine-stimulated gastric acid secretion (1, 2), it increased gastric mucosal blood flow (2), prevented aspirin-induced reduction of gastric mucus (2) and protected the gastric mucosal barrier (3). Therefore, KB-5492 has been considered to enhance gastric mucosal defensive factors. Recently, sigma receptors have been reported to exist in the gastrointestinal tract such as the stomach and duodenum in guinea pigs and humans (4, 5). Some specific sigma-receptor ligands, 1,3-di(2-tolyl)guanidine (DTG), (+)SKF10,047 and JO1784, have been demonstrated to stimulate duodenal HCO₃⁻ secretion in anesthetized rats (6, 7). Furthermore, these sigma ligands showed protective effects on cysteamine induced duodenal ulcer (6, 7). These findings suggest that the sigma receptor plays a pivotal role in the control of mucosal functions. KB-5492 has been reported to have selective affinity for the [³H]DTG-labeled sigma receptor (8). Therefore, the effects of KB-5492 on gastric acid secretion in cysteamine-treated rats and duodenal HCO₃⁻ secretion in normal anesthetized rats were investigated and compared with those of cimetidine.

Male Sprague-Dawley rats weighing 180–300 g (Charles River Japan, Atsugi) were used. The animals were fasted for 24 hr before the experiments. Cysteamine-induced duodenal ulcers were produced in the rats according to the method of Fujii and Ishii (9). Cysteamine hydrochloride (dissolved in saline; Wako, Osaka) was administered to the rats subcutaneously at 400 mg/kg. After 18 hr, the animals were killed. The stomach and duodenum were removed, fixed by inflation with 1% formalin, and then incised along the greater curvature. The area (mm²) of each ulcer formed on the proximal duodenal mucosa was measured under a dissecting microscope, and the sum of the areas of the ulcers in each animal was calculated. Drugs used were KB-5492 (Kanebo, Osaka) and cimetidine (Sigma, St. Louis, MO, USA). Drugs were suspended in 1% gum arabic solutions and administered orally 10 min before cysteamine treatment.

The effects of drugs on gastric acid secretion in cysteamine-treated rats were investigated according to the method of Groves et al. (10). Cysteamine hydrochloride was administered to the rats subcutaneously at 400 mg/kg. After 6 hr, the abdomen was incised under ether
anesthesia and the pylorus was ligated. After 1 hr, the animals were killed. Gastric contents were centrifuged, and the volume of gastric juice was measured. Subsequently, the acidity was determined by titration to pH 7.0 with 0.05 N NaOH. Drugs were administered orally 10 min before cysteamine treatment.

Duodenal HCO$_3$ secretion in anesthetized rats was determined according to the method of Takeuchi et al. (11). The abdomen of the rat was incised under urethane anesthesia (1.25 g/kg, i.p.), and then the stomach and duodenum were exposed. A polyethylene tube was inserted into the duodenal lumen via the forestomach and pylorus and then ligated at the pyloric ring. The other polyethylene tube was inserted into the duodenal lumen at the site just above the outlet of the common bile duct, and there the duodenum was ligated to make a proximal duodenal loop. The duodenal loop was perfused with saline gassed with 100% O$_2$, pH 7.4, at a flow rate of 1 ml/min. The amount of HCO$_3$ secreted into the perfusate was continuously measured by titrating to pH 7.4 with 0.01 N HCl using an automatic pH-stat titrator (AUT-211; TOA Electronics, Tokyo). At least 60 min after the basal secretion had stabilized, drugs were administered intra-intestinally at the site just below the duodenal loop. Statistical significance was determined by one-way analysis of variance followed by Dunnett’s or Duncan’s test. The ED$_{50}$ value was calculated by the least squares method for a regression line.

As shown in Fig. 1, KB-5492, administered orally at 25–200 mg/kg, dose-dependently prevented cysteamine-induced duodenal ulcers in rats with an ED$_{50}$ value of 63 mg/kg. Similarly, cimetidine, administered orally at 25–200 mg/kg, dose-dependently prevented the duodenal ulcers with an ED$_{50}$ value of 40 mg/kg.

As shown in Fig. 2, an ulcerogenic dose of cysteamine hydrochloride (400 mg/kg, s.c.) significantly increased gastric acid secretion in rats. The volume, acidity and acid output of gastric juice secreted from 6 to 7 hr after cysteamine administration were 2.4, 1.2 and 2.9 times greater than those of the control values, respectively. KB-5492, administered orally at 100 and 200 mg/kg, did not affect cysteamine-induced gastric acid hypersecretion. In contrast, cimetidine at 100 and 200 mg/kg significantly and dose-dependently inhibited gastric acid hypersecretion.

As shown in Fig. 3, KB-5492, administered intra-intestinally at 100 and 200 mg/kg, dose-dependently increased duodenal HCO$_3$ secretion in normal anesthetized rats. The amount of HCO$_3$ secreted in the rats treated with 200 mg/kg of KB-5492 was significantly greater than that in the control rats. However, cimetidine at 200 mg/kg did not affect duodenal HCO$_3$ secretion.

In the present study, KB-5492 prevented cysteamine-induced duodenal ulcers in rats; its potency was comparable to that of cimetidine. Groves et al. (10) and Ishii et al. (12) showed that ulcerogenic doses of cysteamine markedly increased the gastric acid secretion in rats, and they regarded this action as a cause of the duodenal ulcers. The present study also showed that an ulcerogenic dose of cysteamine significantly increased the gastric juice volume, acidity and acid output. KB-5492 did not affect cysteamine-induced gastric acid hypersecretion at doses required to prevent the duodenal ulcers, suggesting that KB-5492 prevents cysteamine-induced duodenal ulcers but not by inhibiting gastric acid hypersecretion. In contrast, cimetidine inhibited cysteamine-induced gastric acid hypersecretion at anti-ulcer doses, suggesting that

![Fig. 1. Effects of KB-5492 and cimetidine on cysteamine-induced duodenal ulcers in rats. Cysteamine hydrochloride (400 mg/kg) was administered subcutaneously to the rats, and the animals were killed 18 hr later. Drugs were administered orally 10 min before cysteamine treatment. Each column represents the mean±S.E. of 8 animals. *P<0.05, **P<0.01, significantly different from the control (Dunnett’s test).](image-url)
Cimetidine prevents cysteamine-induced duodenal ulcers by inhibiting gastric acid hypersecretion (13).

On the other hand, Briden et al. (14) and Ohe et al. (15) have proposed that inhibition of duodenal HCO$_3^-$ secretion, an important defensive factor in the duodenal mucosa, is a main causal mechanism by which cysteamine induces the duodenal ulcer. KB-5492, but not cimetidine, increased duodenal HCO$_3^-$ secretion in normal anesthetized rats at doses required to prevent the duodenal ulcers. These findings suggest that KB-5492 prevents cysteamine-induced duodenal ulcers by enhancing the duodenal mucosal defensive mechanism against gastric acid through stimulation of duodenal HCO$_3^-$ secretion.

KB-5492 binds to the $[\text{H}]$DTG-labeled sigma receptor in the guinea pig brain membrane (8) and to both the $[\text{H}]$DTG- and $[\text{H}]$SKF10,047-labeled sigma receptor in porcine gastric fundic mucosa (Y. Harada, unpublished data). On the other hand, cimetidine had a negligible affinity for the $[\text{H}]$DTG binding sites (8). Furthermore, the protective effects of KB-5492 and DTG on ulceration models were antagonized by haloperidol, a sigma-receptor antagonist (16). As above-mentioned, sigma-receptor ligands such as DTG, (+)SKF10,047 and JO1784 have been demonstrated to stimulate duodenal alkaline secretion (6, 7). Accordingly, KB-5492 may stimulate duodenal alkaline secretion and prevent cysteamine-induced duodenal ulcers partly through an interaction with sigma receptors in the duodenum.

In conclusion, KB-5492 prevents cysteamine-induced duodenal ulcers in rats probably by stimulating duodenal HCO$_3^-$ secretion and not by inhibiting cysteamine-induced gastric acid hypersecretion.
REFERENCES


